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Contents

ORIGINAL ARTICLES

(Continued on next page)

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ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

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Under the above caption will be published from time to time comments, criticisms and suggestions on technical procedures; minor contributions such as laboratory aids and short cuts which are not considered sufficiently important to warrant a formal paper; and queries.

Obviously comments and criticisms should be signed; queries should be signed but names will be withheld on request; full credit will be given those who contribute laboratory aids, short cuts and the like.

An attempt will be made to obtain answers from authoritative sources to the queries submitted. It must be emphasized that the views expressed in this department are not the opinions of any official body.

SUBSCRIPTION PRICE $1.50 PER VOLUME

HORTEGA'S SILVER IMPREGNATION STAINS

WM. M. GERMAN

From the Pathological Laboratories of The Good Samaritan Hospital, Cincinnati, Ohio

I have previously presented two groups of Hortega's Silver Impregnation Stains. I present herewith another group, particularly useful in the diagnosis of lesions of the central nervous system.

HORTEGA'S NEUROGLIA STAIN

This method has a very selective action on Neuroglia fibrils and is exceptionally useful in the identification and classification of the glial group of brain tumors and the demonstration of glial proliferation from any cause.

A. *Materials* as before suggested.

B. *Technique:*

1. Tissue fixed in formol.
2. Sections cut by freezing microtome (very thin).
3. Wash sections in distilled water to which have been added a few drops of concentrated ammonium hydroxide.
4. Transfer without further washing to 10 cc. lithium-silver-carbonate solution* plus 5 drops of pyridine, at room temperature.
5. Cover with watch crystal and heat very slowly, 50 to 55 degrees centigrade, over micro-burner until sections are rich brown color.
6. Wash sections rapidly in distilled water.
7. Place sections in 10 per cent formol to reduce silver.
8. Wash sections in distilled water.

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* Lithium-silver-carbonate solution:

Saturated lithium carbonate solution	267 cc.
10 per cent silver nitrate solution	67 cc.
Add ammonia drop at a time until precipitate disappears.	
Distilled water to	1000 cc.

9. Divide sections in two groups, A and B.
   A. 1. Place sections in 5 per cent sodium hyposulphite solution.
      2. Wash in distilled water.
      3. Mount section on slide, drain excess water, blot.
      4. Dehydrate by flooding slide with absolute alcohol, drain, blot.
      5. Clear by flooding slide with creosote, drain, blot.
      6. Mount with balsam and #1 cover-glass.
   B. 1. Put sections in 10 cc., 1:500 gold chloride solution at room temperature.
      2. Without cover, heat over micro-burner, very slowly until the sections are dark.
      3. Wash in distilled water.
      4. Put sections in 5 per cent sodium hyposulphite solution.
      5. Wash in distilled water.
      6. Mount section on slide, drain excess water, blot.
      7. Dehydrate by flooding slide with absolute alcohol, drain, blot.
      8. Clear by flooding slide with creosote, drain, blot.
      9. Mount with balsam and #1 cover-glass.

Results: In Group A all structures are stained various shades of a rich brown and the Neuroglia fibrils are stained black. In Group B the various structures are stained a mahogany red, and Neuroglia fibrils are stained black. The method leaves no doubt as to which are Neuroglia cells. The microglia fibrils are not stained by the method (fig. 1).

HORTEGA'S METHOD FOR MICROGLIA

The microglia, sometimes called the third element or Hortega cells, forms a separate entity in the glial group of cells and requires for its identification a separate staining technique.

A. *Materials* as before suggested.
B. *Technique:*
   1. Tissue fixed in formol-bromide solution* for at least three days (small pieces).
   2. Sections cut by freezing microtome, very thin.

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* Formol-bromide solution:

Ammonium bromide	14 gms.
Conc. formaldehyde	70 cc.
Water	750 cc.

3. Warm sections in same solution, 40 to 50 degrees Centigrade, for 10 minutes.
4. Wash sections in distilled water.
5. Wash in distilled water to which a few drops of ammonium hydroxide have been added.
6. Wash twice in distilled water.
7. Place sections in silver-carbonate solution at room temperature for about 1 minute. Handle sections one at a time.

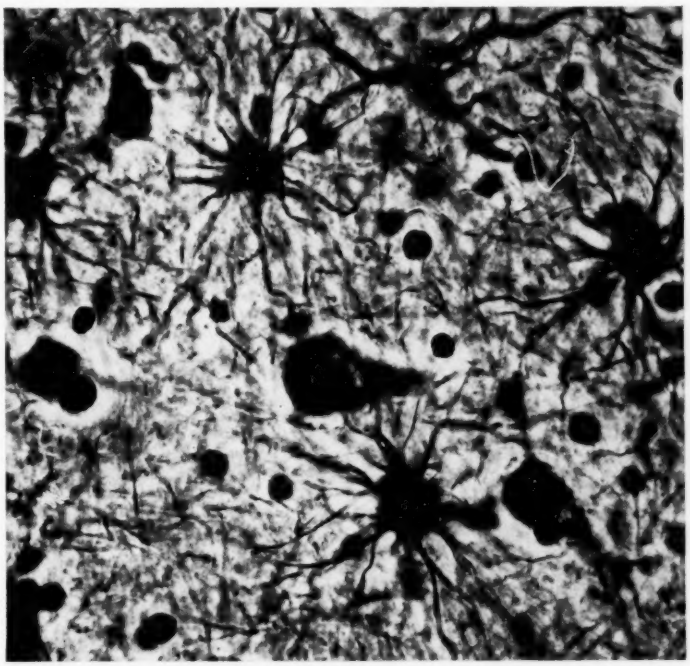

FIG. 1. HORTEGA'S NEUROGLIA STAIN

Photomicrograph of section of cerebrum from a case of paresis. The stain demonstrates glial proliferation. The small cells without processes are microglia and are not stained by this method.

8. Dip section quickly in distilled water and transfer to 1 per cent formol solution to reduce silver. Try several sections and select one which best demonstrates microglia. This method is very rapid and one must work quickly.
9. Wash in distilled water.
10. Divide the sections into two groups, A and B.

A. 1. Place sections in 5 per cent sodium hyposulphite solution.
2. Wash in distilled water.
3. Mount section on slide, drain excess water, blot.
4. Dehydrate by flooding slide with absolute alcohol, drain, blot.
5. Clear by flooding section with creosote, drain, blot.
6. Mount with balsam and #1 cover-glass.

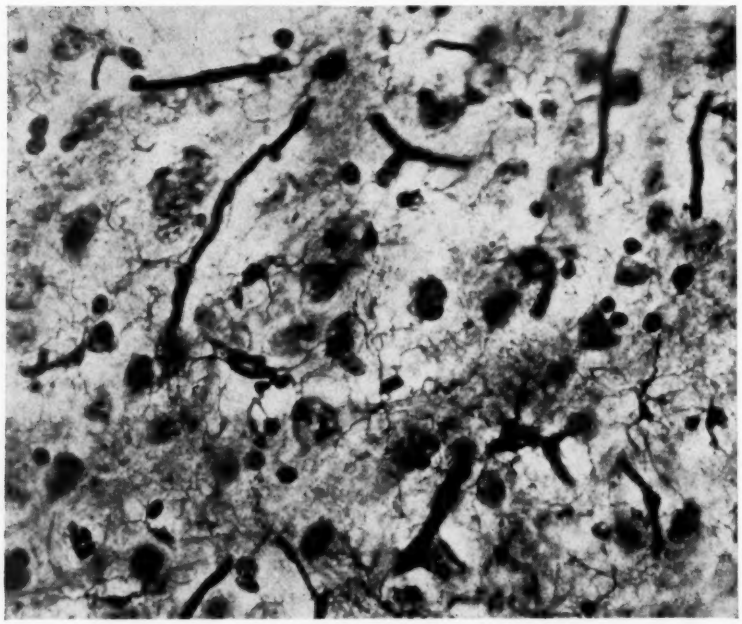

FIG. 2. HORTEGA'S MICROGLIA STAIN

Photomicrograph of a section of the cerebral cortex showing microglia; recognized as minute polyhedral cells with very fine and irregular cell processes. The larger neuroglia cells are to be seen but their processes are unstained by this technique.

B. 1. Place sections in 10 cc. gold chloride solution, 1:500, at room temperature.
2. Heat over micro-burner, very slowly, without cover until the sections darken.
3. Wash sections in distilled water.
4. Place sections in 5 per cent sodium hyposulphite solution.
5. Wash sections in distilled water.
6. Mount section on slide, drain excess water, blot.

7. Dehydrate by flooding slide with absolute alcohol, drain, blot.
8. Clear by flooding slide with creosote, drain, blot.
9. Mount with balsam and #1 cover-glass.

Results: The colors in Series A and B are respectively brown and mahogany red. In both the microglia cells are recognized by a polymorphic nucleus, an irregular cell body and cell processes. The cell processes of the other elements are not stained (fig. 2).

HORTEGA'S IMPREGNATION METHOD FOR NEUROFIBIRILS

A. *Materials* as before suggested.
B. *Technique:*
Series 1.

1. Tissue fixed in formol.
2. Sections cut very thin by freezing microtome.
3. Wash sections in distilled water.
4. Place sections in 2 per cent silver nitrate solution at room temperature.
5. Without cover, heat over micro-burner, very slowly, until the sections are a yellowish color.
6. Wash sections in distilled water.
7. Put sections in 10 cc. silver carbonate solution, at room temperature.
8. Cover with watch crystal and heat very slowly over micro-burner until the sections are rich brown color.
9. Wash sections in distilled water.
10. Place sections in 10 per cent formol to reduce silver.
11. Wash sections in distilled water and divide sections in two groups, A and B.

A. 1. Put sections in 5 per cent sodium hyposulphite solution.
   2. Wash sections in distilled water.
   3. Mount section on slide, drain excess water, blot.
   4. Dehydrate by flooding the slide with absolute alcohol, drain, blot.
   5. Clear by flooding the section with creosote, drain, blot.
   6. Mount with balsam and #1 cover-glass.

B. 1. Put sections in gold chloride solution, 1:500, at room temperature.
   2. Without cover, heat over micro-burner very slowly until the sections are very dark.
   3. Wash sections in distilled water quickly.
   4. Place sections in 5 per cent sodium hyposulphite solution.
   5. Wash sections in distilled water.

6. Mount section on slide, drain excess water, blot.
7. Dehydrate by flooding slide with absolute alcohol, drain, blot.
8. Clear by flooding slide with creosote, drain, blot.
9. Mount with balsam and #1 cover-glass.

Series 2.

1. Sections cut by freezing microtome, very thin.
2. Wash sections in distilled water.
3. Place a few sections in 10 cc. alcohol plus 30 drops ammonium hydroxide at room temperature.
4. Warm very gently for 10 minutes.
5. Wash in distilled water.
6. Put sections in 10 cc. of 2 per cent silver nitrate at room temperature.
7. Without cover, heat slowly over a micro-burner until the sections are yellowish color.
8. Wash sections in distilled water.
9. Place sections in 10 cc. silver-carbonate solution at room temperature.
10. Cover with a watch crystal and heat very slowly over a micro-burner with occasional rotation, until the sections are a rich brown color.
11 Wash sections in distilled water.
12 Place sections in 10 per cent formol to reduce the silver.
13. Wash in distilled water and divide the sections in groups, A and B.

A. 1. Place sections in 5 per cent sodium hyposulphite solution.
   2. Wash in distilled water.
   3. Mount section on slide, drain excess water, blot.
   4. Dehydrate by flooding slide with absolute alcohol, drain, blot.
   5. Clear by flooding slide with creosote, drain, blot.
   6. Mount with balsam and #1 cover-glass.

B. 1. Place sections in 10 cc. gold chloride solution, 1:500, at room temperature.
   2. Heat over micro-burner, very slowly, without cover, until the sections become very dark.
   3. Wash sections quickly in distilled water.
   4. Place sections in 5 per cent sodium hyposulphite solution.
   5. Wash sections in distilled water.
   6. Mount section on slide, drain excess water, blot.
   7. Dehydrate by flooding slide with absolute alcohol, drain, blot.
   8. Clear by flooding slide with creosote, drain, blot.
   9. Mount with balsam and #1 cover-glass.

The above outlined technique visualizes certain epithelial neurofibrils and cell processes which are not demonstrated with ordinary staining technique. The fibrils and cell processes are

stained black with a brown or mahogany red background as the case may be, whether silver alone or the superimposed gold is used (fig. 3).

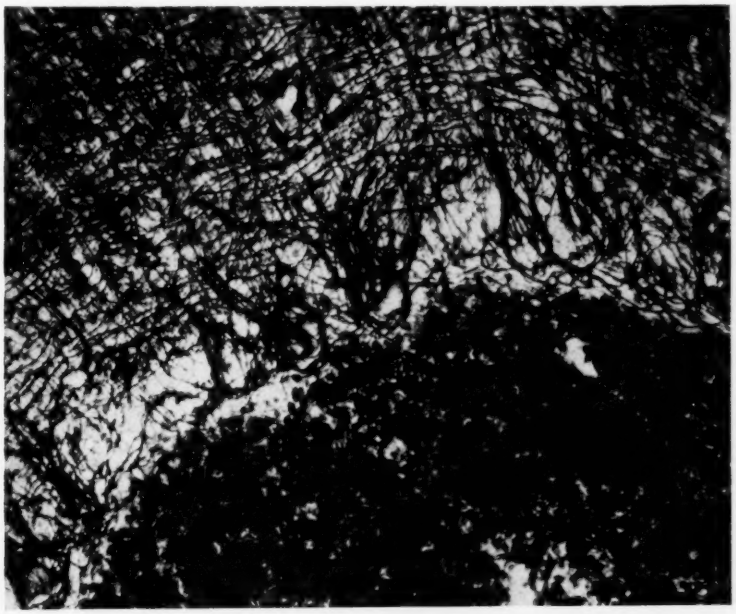

FIG. 3. HORTEGA'S STAIN FOR NEUROFIBRILS

Photomicrograph of section of Cerebellum showing Basket cells whose processes run horizontally and dip to surround the Purkinje cells.

HORTEGA'S MODIFICATION OF THE SILVER TANNATE METHOD OF ACHUCARRO

A. *Materials* as before suggested.

B. *Technique:*

1. Tissue fixed in 10 per cent formol solution for at least one month.
2. Sections cut very thin by freezing microtome.
3. Wash sections in distilled water.
4. Transfer sections to 3 per cent tannic acid solution and heat gently for 10 minutes.
5. Wash in distilled water to which have been added a few drops of ammonium hydroxide, until the return of the flexibility of the sections.

6. Pass through three treatments of Bielschowski's silver solution* at room temperature, five minutes in each solution, until the sections are dark.
7. Wash sections in distilled water.
8. Transfer sections to gold chloride solution, 1:500, at room temperature.
9. Without cover, heat slowly over micro-burner until sections are dark.

FIG. 4. HORTEGA'S MODIFICATION OF SILVER TANNATE METHOD

Photomicrograph of section of Cerebral Cortex illustrating use of the method for the visualization of cell processes of Pyramidal Neurones.

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* Bielschowski's Silver Solution:

10 per cent silver nitrate ..... 5 cc.
40 per cent sodium hydroxide solution ..... 6 drops

Wash the resultant precipitate 6 times, then add a little water and ammonium hydroxide, drop by drop, until the precipitate is completely dissolved.

Distilled water ..... to 25 cc.

(For making 500 cc. of same use following proportions:

10 per cent silver nitrate ..... 100 cc.
40 per cent sodium hydroxide ..... 7 cc.
Ammonium hydroxide to dissolve the precipitate.
Make up with distilled water to volume 500 cc.)

10. Wash quickly in distilled water.
11. Put sections in 5 per cent sodium hyposulphite solution.
12. Wash in distilled water.
13. Mount section on slide, drain excess water, blot.
14. Dehydrate by flooding slide with absolute alcohol, drain, blot.
15. Clear by flooding slide with creosote, drain, blot.
16. Mount with balsam and #1 cover-glass.

This method visualizes mitochondria, cellular fibrils and certain cell processes not revealed by the ordinary staining methods (fig. 4).

I have presented the foregoing groups of Hortega's Silver Impregnation tissue stains, that they may be in such a place and form that they can be of general use. I wish to encourage their more widespread use. They have each their special use and purpose. They are not difficult either to learn or carry out and make use of reagents readily available and which do not deteriorate. I claim no originality for the methods and in conclusion wish to pay tribute to Dr. P. Del Rio Hortega, a brilliant teacher and a delightful host.

RAPID HEATING OF SERUM FOR THE SLIDE TESTS FOR SYPHILIS*

Preliminary Report†

BENJAMIN S. KLINE and DOROTHY K. LLOYD

From the Laboratory Department, Mount Sinai Hospital

Sera heated for 4 minutes at 61°C. to 62°C. give results in the microscopic slide precipitation tests for syphilis that are practically identical with those obtained with the sera after 30 minute heating at 56°C. Such rapid preparation of serum is especially valuable whenever an immediate blood test for syphilis is indicated as in selecting a blood donor just prior to transfu-

* Received for publication June 17, 1938.

† *With the assistance of funds available to one of us as Special Consultant, U. S. Public Health Service.*

Read before the Seventeenth Annual Convention of The American Society of Clinical Pathologists held at San Francisco, California, June 9–11, 1938.

TABLE 1

RESULTS OF EXCLUSION SLIDE TESTS ON SERA UNHEATED, RAPIDLY HEATED AND ROUTINELY HEATED

DATE, 1938	SERUM NO.	MINUTES SERUM HEATED													
		0	30	1½			3			5			8		
		TEMP. °C.													
			56	60	61	62	60	61	62	60	61	62	60	61	62
3/24	27	−	++++			++			++++			++++			++++
	3	−	++++			++++			++++			++++			++++
	19	+	++++			++++			++++			++++			++++
	10	−	+			±			+			+			++
	9	±	++			++			+++			+++			
	11	±	++			++			++			+++			
	1	+	+++			+++			+++			+++			+++
3/31	20	−	++++		++++			++++			++++			++++	
	15	±	+		±			+			+			+	
	9	−	±		±			++			+			±	
	14	−	±		±			±			±			±	
	12	−	±		±			±			±			±	
4/2	12	−	++++	++++			++++			++++			+++		
	5	+	++++	++++			++++			++++			++++		
	6	++	++++	++++			++++			++++			++++		
	19	++++	++++		++++			++++			++++			++++	
	15	++	+++		+++			+++			+++		+++		
	13	+	+	+			+			+			±		
	3	±	±	±			±			±					

TABLE 2

Results of Exclusion Slide Tests on Sera Unheated, Rapidly Heated and Routinely Heated

Date, 1938	Serum No.	Minutes serum heated: 0	30	2				3				4				5				6			
		Temp. °C.:	56	60	61	62	63	60	61	62	63	60	61	62	63	60	61	62	63	60	61	62	63
4/13	18	−	+++	+++								++++								+			
	19	−	+++	+++								+++											
	8	−	±		±								±								±		
	13	−	++++	++++								++++								++++			
	10	+	++++		++++								++++								++++		
4/15	16	−	+++		++								+++								+++		
	14	−	++++		+								++++								++++		
4/22	12	±	+				−								±								+
5/2	15	+	+++	+++									+++								+++		
5/4	5	−	+++	+++									+++								+++		
5/6	44	−	+++				+++				+++				+++				+++				
	42	−	++++				+++				++++				++++				++++				
5/9	30	++	+++		+++				+++				+++				+++						
	32	±	+		±				±				+				+						
5/11	9	+	++		+				++				++				++						

TABLE 3

RESULTS OF DIAGNOSTIC SLIDE TESTS ON SERA UNHEATED, RAPIDLY HEATED AND ROUTINELY HEATED

DATE, 1938	SERUM NO.	MINUTES SERUM HEATED																					
		0	30	2				3				4				5				6			
		TEMP. °C.																					
			56	60	61	62	63	60	61	62	63	60	61	62	63	60	61	62	63	60	61	62	63
4/13	11	−	+		+								+								±		
	18	−	+	+								++								+			
	19	−	±	−								±											
4/15	14	−	+++		±								+++								++++		
	21	−	+		±								+								+		
4/22	25	−	+			+								+								+	
	35	+	+++			+++								+++								+++	
	14	−	±				±								+								+
4/28	37	−	±	+++				+++				−				−							
4/29	17	−	+			+				+				±				±					
	18	−	−			−				−				±				±					
5/2	16	−	+++	+				+++				+++				+++							
	18	±	+++	+				+++				+++				+++							
	13	−	±				−				−				±				+				
	2	−	++			+				+				++				++					
	9	−	++			+				++				++				++					
5/6	17	+	+++	++				+++				+++				+++							
	42	−	+++				+				+++				++++				++++				
5/13	28	±	++		+				+				++				++						

sion. Rapid heating may eventually replace the routine 30 minute heating of sera.

Last year, Strauss[1] reported that the heating of serum for 3 minutes at 60°C. to 63°C. has the same effect with respect to the Eagle flocculation test as heating for 30 minutes at 56°C. Following the appearance of this report, Rein and Hazay[2] made a similar comparative study of the effect of rapid and routine heating of over 2,000 sera for the microscopic slide precipitation tests for syphilis. They found that heating for 3 minutes at 60°C. to 63°C. was the equivalent in the slide tests also of heating for 30 minutes at 56°C. Because of the great importance for the slide test technic of this detail, it was decided that additional studies of the same kind should be done to aid in establishing its value. Accordingly, during the past year we have made a comparative study of rapid and routine heating using serum samples of no less than 0.75 cc. and most frequently of 1 cc. or more. Representative protocols of the results are shown in tables 1, 2 and 3.

TABLE 4

HEATING EQUIVALENCE (30 MINUTES AT 56°C.) OF SERA FOR SLIDE TESTS

	REIN AND HAZAY	KLINE AND LLOYD
Minutes at 56°C.	10	15
Minutes at 60°C.–63°C.	3–5 (3)	3–5 (4)
Minutes at 68°C.–70°C.	1	1
Seconds at 100°C.	7	8

From the results, as shown in the tables, it is concluded that sera heated for 4 minutes at 61°C. to 62°C. give results in the microscopic slide precipitation tests for syphilis that are practically identical with those obtained with the sera after 30 minute heating at 56°C.

Rein and Hazay established not only the heating equivalence of serum for the slide tests at 60°C. to 63°C. but also established it at 56°C., at 68°C. to 70°C. and at 100°C. (in boiling water). Our findings, to date, at these temperatures (in 4,024 tests of 490 sera) are essentially the same (see table 4).

The small number of sera heated for 1 minute at 69½°C. thus far tested gave results approximating but not quite equalling those of sera heated for 30 minutes at 56°C. Further comparative study of such sera is being conducted and it is possible that sera heated at a little higher temperature for 1 minute or for a little longer time at the same temperature may give thoroughly satisfactory results.

Sera coagulate in about 80 minutes at 61°C.; 4 minutes at 69°C.; 10 seconds at 100°C.

CONCLUSIONS

Sera heated for 4 minutes at 61°C. to 62°C. give results in the microscopic slide precipitation tests for syphilis that are practically identical with those obtained with the sera after 30 minute heating at 56°C. Such rapid preparation of serum is especially valuable whenever an immediate blood test for syphilis is indicated as in selecting a blood donor just prior to transfusion. Rapid heating may eventually replace the routine 30 minute heating of sera.

REFERENCES

(1) STRAUSS, J. H.: Modification of the Eagle Flocculation Test for Syphilis. Am. J. Syph. Gon. and Ven. Dis. **21**, 406, 1937.

(2) REIN, C. R. AND HAZAY, C. E., New York, N. Y.—Personal communications.

THE DETERMINATION OF BLOOD GLUCOSE WITH 2,6 DICHLORPHENOLINDOPHENOL*

E. M. ABRAHAMSON

From The Jewish Hospital of Brooklyn

Of all the constituents of blood, glucose is determined more frequently than any other. In fact, many physicians find it necessary to perform the analysis in their offices. Other substances are, as a rule, determined only in hospitals with their complete laboratory facilities.

* Received for publication November 14, 1938.

There are many methods available based on the reducing property of glucose which may be classified as gasometric, colorimetric, titrimetric, and gravimetric. None hitherto published is ideally suitable for office work, for the cost of the apparatus is likely to prove prohibitive unless it is also used for other determinations.

In the method proposed, advantage is taken of the fact that there are on the market tablets of 2,6 dichlorphenolindophenol which are made up so accurately that the only standard solution needed for the determination can be prepared without costly apparatus.

Principle. The glucose in a Folin-Wu blood filtrate reduces ferricyanide in alkaline solution. After acidification, the ferrocyanide produced is titrated with a standard dye solution that is its own indicator.

Reagents. 10 per cent sodium tungstate, according to Folin and Wu[1].

⅔ N sulphuric acid (Folin-Wu).

Tungstic acid. Mix 1 volume of each of the above with 8 volumes of water. The mixed reagent should be prepared at least fortnightly.

0.005 N potassium ferricyanide. Dissolve 1.65 grams of purest potassium ferricyanide in water and dilute to a liter. The solution must be free from ferrocyanide (a test portion should not give a green color with ferric chloride) and from ferric salts (a test portion should not give a green or blue color with ferrocyanide solution). The reagent is kept in a dark bottle in a cool place.

0.04 N sodium carbonate. Dissolve 2.1 grams of fused sodium carbonate in water and dilute to a liter. Preserve the solution in a waxed bottle.

The ferricyanide and sodium carbonate may be combined into one reagent by dissolving 1.65 grams of potassium ferricyanide and 10.6 grams of fused sodium carbonate in water and diluting to a liter. The mixture should be tested for impurities as for the ferricyanide solution and kept in a waxed brown bottle. It is not quite as stable as the separate reagents.

Glacial acetic acid.

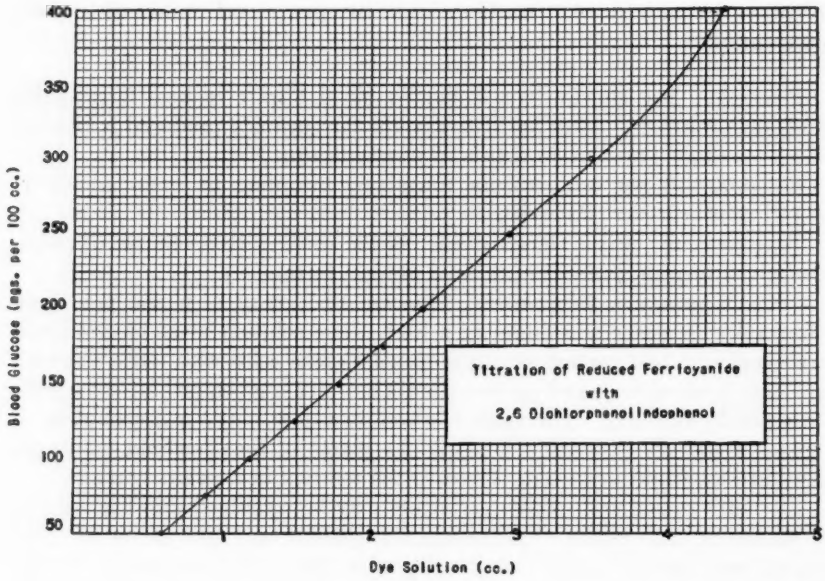
2,6 dichlorphenolindophenol. Dissolve 1 tablet (Hoffman-LaRoche) in water and dilute to 10 cc. The solution should be prepared fresh on the day it is used.

Apparatus. Conical tipped centrifuge tube, 0.1, two 1, 2, 3, and 5 cc. pipettes, boiling water bath, 6″ x ¾″ flat bottomed test tubes (so called specimen tubes), 10 cc. graduated flask or cylinder, 5 cc. microburette graduated to 0.01 or 0.02 cc. [For most clinical purposes an ordinary burette or Mohr pipette graduated to 0.1 cc. is quite satisfactory].

Procedure. (1) Place a dye tablet in a 10 cc. graduated flask or cylinder and add about 8 cc. of water. Shake until the tablet is completely dissolved and dilute to the mark with water.

(2) Place 2 cc. of tungstic acid in a centrifuge tube. Make a lancet wound in the finger tip and draw up the blood in a 0.1 cc. pipette calibrated "to contain." Discharge the blood into the tungstic acid. Rinse the pipette twice by drawing up the solution and blowing it out. Finally, place the tip of the pipette at the bottom of the tube and blow a gentle current of air through the liquid to secure an homogeneous mixture. When the coagulum has turned brown, centrifuge. A wax cup is advantageously used to catch the blood from the wound[2].

(3) Using care not to disturb the precipitate, pipette 1 cc. of the clear supernatant fluid into a flat bottomed test tube.



(4) Add 1 cc. of potassium ferricyanide reagent and 5 cc. of the sodium carbonate solution. If the mixed reagent is used add 1 cc. and 5 cc. of water.

(5) In a similar tube, prepare a blank with the reagents but no filtrate.

(6) Place the unknown in boiling water for exactly 15 minutes. Cool the tube by immersing it in cold water.

(7) Add 3 cc. of glacial acetic acid to the unknown and blank. Allow the tubes to stand until the CO_2 is expelled.

(8) Hold the unknown and blank next to each other and add the dye to the former from a burette or Mohr pipette until the pink color of the dye in acid solution can be detected. The two tubes are observed by looking down through them onto a sheet of white paper. At the end point the unknown and blank contain the same amount of ferricyanide and the difference in shade is easily

detected. The comparison may be facilitated by wrapping each tube in white paper to shut out the light coming from the sides.

Calculation. If the volume of dye solution is less than 3.50 cc., multiply it by 85 to obtain the blood sugar in mgs. per 100 cc. For volumes greater than 3.50 cc. use the graph.

Testing the Reagents. About once a month prepare two blanks and heat one of them. Titrate the heated blank as for an unknown. The first drop of dye should give a noticeable pink shade. If a significant amount of dye is required, the reagents should be discarded.

Standardization. Dilutions of standard 1 per cent glucose to correspond with different blood sugar values were prepared and each was analyzed in triplicate. The dye solution was prepared by dissolving 10 tablets in water and diluting to 100 cc.

BLOOD SUGAR	CC. DYE SOLUTION			AVERAGE
50	0.58	0.61	0.61	0.60
75	0.91	0.87	0.90	0.89
100	1.22	1.17	1.18	1.19
125	1.49	1.47	1.52	1.49
150	1.80	1.81	1.75	1.78
175	2.12	2.08	2.04	2.08
200	2.33	2.32	2.40	2.35
250	2.98	2.90	2.90	2.93
300	3.44	3.46	3.54	3.48
350	3.96	3.97	4.00	3.98
400	4.40	4.39	4.35	4.38

Four separate solutions were prepared by dissolving 1 tablet in water and diluting to 10 cc. They were used to check the "200" standard. The volumes of dye solution used were 2.35, 2.25, 2.31 and 2.39 cc.

The method was checked against the Folin-Wu colorimetric determination using four blood samples.

Folin-Wu	235	117	228	290	87 mgs. per 100 cc.
Dye	228	111	230	278	89 mgs. per 100 cc.

Comment. This method has the advantage of not requiring analytical accuracy in preparing the reagents. A photographic scale costing $4.50 was used in making up all the solutions. The glassware is quite inexpensive and the cost may be further reduced by using a less accurate burette than was employed in determining the graph. Since an error of even 10 per cent is

permissible for clinical purposes a Mohr pipette may be used. The entire outfit can be assembled at a cost under $15.00.

The end point requires a little practice. There is no great difficulty in detecting the difference in shade between the unknown and blank. The difference in volume between the blank and unknown does not affect the accuracy since both contain the same amount of ferricyanide at the end point. If, for example, one tube contains twice as much liquid as the other, the concentration of ferricyanide in it will be half as much as in the other. However, the depth of fluid under observation will be twice as great and these two effects cancel each other. It is for this reason that flat bottomed tubes are used.

The wax cup is prepared by pouring melted paraffin into the depressions of a muffin tin. When the wax solidifies, the shrinkage produces a concave surface on which the blood can be caught. The blood does not clot readily on the wax.

I wish to express my gratitude to Hoffman-LaRoche, who kindly furnished the tablets.

REFERENCES

(1) Folin and Wu: J. Biol. Chem., 1919, **38,** 81.
(2) Abrahamson: Science, 1937, **86,** 201.

SIMPLE APPARATUS AND TECHNIQUE FOR GAS ANALYSIS*

SAMUEL BERG

From the Laboratory of the Newark City Hospital

The need for determining the oxygen and carbon dioxide content of the pleural air in a patient with a spontaneous pneumothorax in order to diagnose the presence of a pleuro-pulmonary fistula led to the construction of an apparatus which can be assembled very easily and quickly with articles available in every laboratory. The technique of analysis is very simple, and is accurate enough for most clinical work. It will be found of

* Received for publication August 1, 1938.

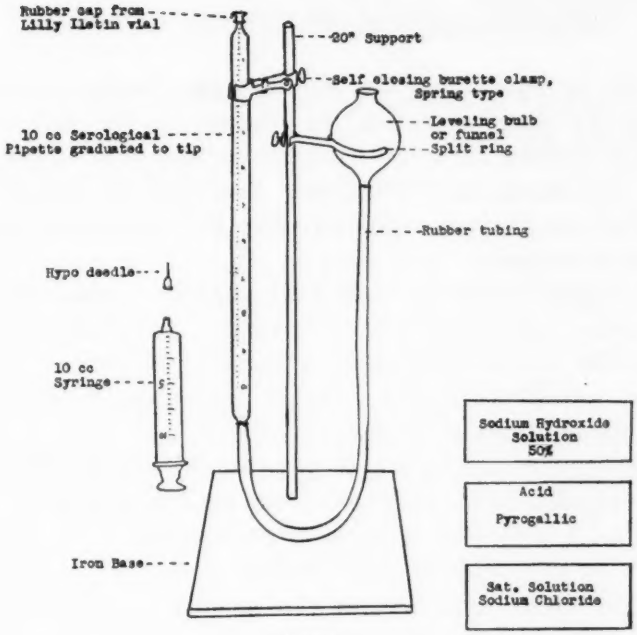

Rubber cap from Lilly Iletin vial
20" Support
Self closing burette clamp, Spring type
Leveling bulb or funnel
Split ring
10 cc Serological Pipette graduated to tip
Rubber tubing
Hypo needle
10 cc Syringe
Iron Base
Sodium Hydroxide Solution 50%
Acid Pyrogallic
Sat. Solution Sodium Chloride

FIG. 1

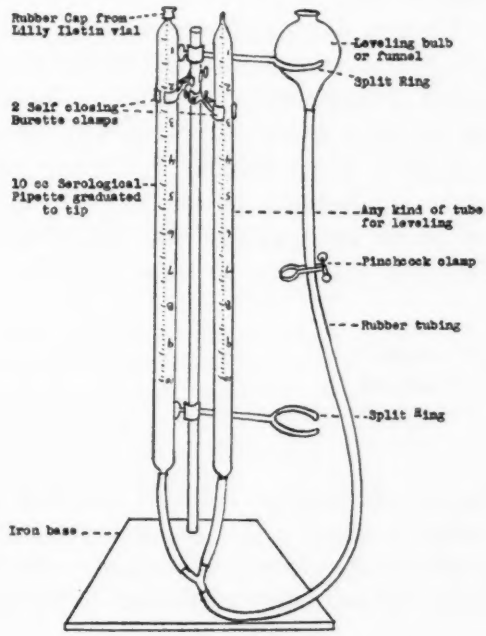

Rubber Cap from Lilly Iletin vial
Leveling bulb or funnel
Split Ring
2 Self closing Burette clamps
10 cc Serological Pipette graduated to tip
Any kind of tube for leveling
Pinchcock clamp
Rubber tubing
Split Ring
Iron base

FIG. 2

value also in determining the size of a pneumothorax space, the presence of a closed bronchus to a lung cavity, the carbon dioxide content of alveolar air and the oxygen content of air in pneumonia tents. Henderson and Greenberg[1] described an equally simple method of gas analysis using an all-glass Luer syringe and a few mechanical gadgets.

The original assembly is shown in figure 1, which is so clear as to make unnecessary any directions. A modified assembly, making for easier manipulation because of more convenient pressure adjustments, yet quickly assembled from common laboratory equipment, is shown in figure 2.

The chemicals used are a 50 per cent sodium hydroxide solution, pyrogallic acid (obtained from the photographic department) and a saturated solution of sodium chloride. The latter is used for gas replacement instead of mercury. Peters and Van Slyke[2] state that "water can usually be used as fluid in contact with gas mixtures containing O_2, N_2 and CO, which are not very soluble, if the contact is not prolonged more than a working day. Carbon dioxide, however, is so soluble in water that contact of respired air with water can not be permitted for even a brief period of time without significant absorption of CO_2. If, however, the water is nearly saturated with calcium chloride, and slightly acidified, the solubility coefficient of this gas at room temperature is lowered from about 1.0 to 0.06." Carbon dioxide has at room temperature the following approximate solubility coefficients, according to these authors:

Water	0.90
Ammon. Sulph. solution	0.22
Sodium chloride, sat. sol.	0.24
Calcium chloride, sat. sol.	0.07
Glycerol	0.31

Because contact of calcium chloride solution with sodium hydroxide solution as used in this apparatus results in a heavy precipitate, a saturated solution of sodium chloride was used instead; but since the solubility coefficient of the latter for CO_2 is moderate in degree, contact between gas and salt solution

should be a matter of only a few moments, just long enough to permit the gas to come to the temperature of the room and apparatus.

TECHNIQUE

When the apparatus is set up, the leveling bulb is half filled with salt solution at room temperature. The hypo needle, attached to the syringe, is pushed for a short distance through the rubber cap and the air trapped in the pipette is drawn out so that salt solution fills the pipette completely. The gas for analysis is now drawn into the syringe, which should be dry and sterile when tapping body cavities. The gas, in any amount up to 10 cc., is then injected into the pipette through the rubber cap. After several minutes for temperature equilibrium, and after adjusting the leveling bulb and pipette so that the fluid levels in each are on the same plane, the amount of gas in the pipette is read off by its calibrations.

In using the apparatus shown in figure 2, the leveling bulb is placed in the upper split ring when the fluid in the leveling tube must be raised, and in the lower split ring when it must be dropped.

Determination of carbon dioxide content of gas

Small quantities, about 1 cc., of sodium hydroxide solution are injected into the pipette through the rubber cap so that the solution runs down its side. A 2 cc. syringe is very convenient for this purpose. This is repeated every few minutes until there is no further absorption of gas. After adjusting the leveling bulb, the reading of gas content is taken and the difference between the two readings is the amount of carbon dioxide absorbed.

$$\text{Per cent } CO_2 = \frac{\text{Amount of gas absorbed}}{\text{Original amount of gas}}$$

Determination of oxygen content of gas

If the gas to be analyzed contains carbon dioxide, its amount must be determined first in the manner described above. The procedure is repeated for oxygen absorption, using as the absorbent a freshly prepared solution of pyrogallic acid in 50 per cent sodium hydroxide solution. About 1 gm. of the powder, or the amount that can be piled up on a twenty-five cent coin, is dissolved in about 10 cc. of the latter and is ready for use when the color has become very dark. Accurate measurements are not necessary. About 1 cc. of this solution is injected into the pipette every few moments. Enough time should be allowed for complete absorption of oxygen, which is a slower process than CO_2 absorption. The percentage of oxygen is computed by the formula already given.

If both carbon dioxide and oxygen are present, it must be remembered that

in computing the percentage of oxygen, the denominator is the *original* amount of gas, and *not* the amount present after CO_2 has been absorbed.

Suggestions

A small drop of fluid persists in remaining in the tip of the pipette just beneath the rubber cap regardless of tapping or shaking. Since this is practically constant in amount, it may be disregarded in measurements.

Allow sufficient time, before making readings, for the liquid to drain down the sides of the pipette. If glassware and tubing are not scrupulously clean, drops of liquid will persist in clinging to the sides of the tube. These may be washed down by tilting the tube almost to horizontal and turning upright rapidly.

The level of the liquid in the pipette and in the leveling bulb should not vary too much at any time in order to avoid leakage past the rubber cap, which is held in place only by friction.

A funnel may be used as a leveling bulb.

A 25 cc. Mohr pipette with its uncalibrated tip cut off to the first calibration mark and capped with a rubber cap from a Squibb Insulin vial can be used for analysis of larger amounts of gas.

The sodium hydroxide solution, being heavier than the salt solution, gravitates through the latter and becomes diluted. Gas absorption can be accelerated by removing the pipette from the clamp and tipping it to almost horizontal to effect a large gas-liquid interphase.

REFERENCES

(1) Henderson, Y., and Greenberg, L. A.: Gas Analysis with an All-Glass Syringe for Pneumonia Tents. J. A. M. A. **96:** 1474–1475 (May 2) 1931.

(2) Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. II, page 85. Williams & Wilkins Co., 1932.

THE SURVIVAL OF MICROORGANISMS IN FIXED AND STAINED PREPARATIONS*

HARRY E. MORTON

Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Most of us were taught, and many still teach, that non-spore-forming micro-organisms are no longer viable after being smeared, air-dried and fixed by passing three times through the flame of

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a Bunsen burner; that the micro-organisms are certainly no longer viable after treatment of the fixed preparation by the usual bacteriological stains. It was a great surprise, therefore, to have attention called to an article by Thurn in which it was shown that micro-organisms are not necessarily killed in fixed or fixed and stained preparations.

Thurn[1] found that preparations of *Micrococcus pyogenes aureus, Bacterium typhi, Bacterium coli commune, Bacillus anthracis, Vibrio cholerae, Corynebacterium diphtheriae* and *Saccharomyces cerevisiae,* smeared onto glass slides and fixed by passing three times through the flame still contained viable organisms. Some eighteen preparations of pathogenic and non-pathogenic organisms were not alive after staining by the Gram technic. Only *B. anthracis* and *B. mesentericus,* of the organisms tested, survived one and three minutes treatment with carbol-fuchsin and methylene blue for five minutes each.

EXPERIMENTAL

Smears were made on sterile ½ inch circles, No. I red label, non-corrosive cover-slips and handled under strictly aseptic conditions. Freshly prepared smears on cover-slips were immediately dropped into flasks of infusion broth (50 cc. broth in a 125 cc. Erlenmeyer flask) as controls. All flasks showed characteristic growth of the test organisms after a suitable incubation period. All other smears were fixed by passing three times through the flame of a Bunsen burner. Fixed preparations were also dropped into flasks of infusion broth and incubated. Preparations of *Staphylococcus aureus, Staphylococcus albus, Pneumococcus type I, Streptococcus fecalis* and *Mycobacterium phlei,* among some forty preparations tested, were found to survive such fixation.

Fixed smears were treated with basic fuchsin[2] for one minute, Ziehl's carbol-fuchsin[2] for 30 seconds, Hucker's gentian violet[2] for one minute, aqueous safranin[2] for one minute, Mallory's acetic methylene blue[2] for 15 minutes, Loeffler's alkaline methylene blue[2] for 15 minutes and by Hucker's modification of the Gram technic[2].

None of the micro-organisms survived the treatment with carbol-fuchsin, Hucker's modification of the Gram stain or Mallory's acetic methylene blue.

Two of some forty preparations survived treatment with basic fuchsin, one with Hucker's gentian violet, four with aqueous safranin and two with Loeffler's alkaline methylene blue. Nine preparations did not survive fixation by heat or any of the staining procedures.

Had we used glass slides instead of cover-slips, undoubtedly more of the

micro-organisms would have survived the flame-fixation as the thin cover slips become much warmer than do glass slides which receive similar treatment.

CONCLUSIONS

Some micro-organisms survive fixation onto glass slides and cover slips by passing three times through the flame. In addition certain organisms survive treatment with basic fuchsin, Hucker's gentian violet, aqueous safranin and Loeffler's methylene blue.

More care should be used in the handling of stained preparations in the laboratory, as the fact that the preparation has been stained is no indication that the organisms are necessarily killed.

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(2) Manual of the Methods for Pure Culture Study of Bacteria, Society of American Bacteriologists, 1937.

A NOTE ON THE USE OF "DOUBLE POURED" BLOOD PLATES IN THE EXAMINATION OF THROAT AND NOSE CULTURES FOR HEMOLYTIC STREPTOCOCCI*

C. A. PERRY, AND ELIZABETH PETRAN

From the Bureau of Bacteriology, Maryland State Department of Health, Baltimore

In a public health laboratory a great many throat and nose cultures are received in order to determine the type of bacteria associated with infections of the upper respiratory tract. One type of bacteria found associated with such infections more than any other, is the hyemolytic streptococcus. It is generally accepted that "poured" blood agar plates are more satisfactory for the detection of hemolytic streptococci than "streaked" plates. When poured plates were first used in this laboratory several years ago, a practical problem of how to secure well isolated colonies was immediately encountered. Various methods of doing this were tried but the results of most of these were not

* Received for publication October 15, 1938.

very satisfactory. When blood plates are made after emulsifying the cultures in broth or salt solution it is very difficult, if not impossible, to decide upon a dilution factor and amount of inoculum which will be applicable to a specimen regardless of the amount of original material present, unless several plates are poured for each specimen. This, of course, is not practical in a laboratory making a large number of these determinations. The method described below, however, has been found satisfactory and since an interest in it has been shown by numerous outsiders together with requests for a written procedure, it is presented here.

Plates of infusion agar are inoculated with the swab from the site of infection or from the growth that has occurred in transit on slants of tellurite agar.† The plate is carefully streaked in the ordinary manner to secure well isolated colonies. About 7 cc. of blood agar (10 per cent sterile sheep blood in infusion agar) is then poured over the streaked plate and the plate rotated once or twice to mix the inoculum with the melted blood agar. The medium is allowed to harden and the plates incubated in the usual manner for from 18–24 hours.

If the inoculum is heavy, isolated colonies will only be found in the thinly streaked section, while if the inoculum is light they will be on the original site of inoculation or in the immediate vicinity. Slight rotation of the melted blood agar usually brings a number of colonies to the surface. Surface or deep colonies may be fished for further identification. An idea may be gained as to the relative numbers of hemolytic streptococci present. In many cases of scarlet fever and tonsillitis, etc., practically pure cultures of hemolytic streptococci are obtained.

The plates of infusion agar are easier to store than if made of blood agar, save blood, provide a satisfactory base for streaking and make hemolysis easier to read due to the thinner layer of blood agar.

REFERENCE

WADSWORTH, A. B.: Standard Methods of New York State Department of Health, Williams & Wilkins Co., Baltimore, pp. 101 and 111, 1927.

† Tellurite agar slants are sent into the field and inoculated by the physician when the culture is taken. This medium has been found to be very effective as a preservative medium for streptococci as well as diphtheria bacilli.

AZOCARMINE STAIN FOR BONE MARROW*

MARTHA B. RALSTON AND ARTHUR H. WELLS

From the Laboratory of St. Luke's Hospital, Duluth, Minnesota

Azocarmine in combination with any of several blue dyes has proven to be a most satisfactory stain for differential study of bone marrow. Student technicians with a minimum of training in micro-technique have produced uniformly good results with this stain. The wide contrast of vivid colors in the various myeloid elements leaves but a negligible percent of unclassifiable cells. The shrinkage due to fixation is more than compensated for by the assurance that all cells are present in their in vivo relationship, a virtue not applicable to the results of aspiration or imprint methods.

The 5 per cent glacial acetic acid in Zenker's solution decalcifies the medullary bone during over night fixation. The tissue is then washed in tap water for 24 hours and prepared in paraffin. We routinely use a rapid acetone and benzene method of dehydration, however, other methods have worked as well. The paraffin is removed with xylol from the sections previously cut at not more than four microns, then they are carried through the alcohols to water.†

STAINING TECHNIQUE

(1) Heat the azocarmine solution (a) to 56°C. in the paraffin oven and set the slides in it for 20 minutes.

(2) Wash in several changes of distilled water.

(3) Decolorize and differentiate in aniline alcohol (b) followed by acid alcohol (c) until sections are rose red, a matter of about 30 to 90 seconds.

(4) Wash in distilled water.

(5) Stain for from 10 to 15 seconds with toluidine blue (d).

(6) Wipe off excess water and dehydrate quickly in absolute alcohol.

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* Received for publication August 1, 1938.

† Zenker's fixative forms crystals in the tissues which must be removed by placing the sections in Lugol's solution for about 2 minutes. Then washing in 2 per cent aqueous solution of sodium thiosulphate until all of the iodine stain has been removed. After washing for 5 minutes in running tap water, place sections in distilled water.

(7) Clear in 2 changes of xylol.
(8) Mount in gum damar or balsam.

Reagents

(a) Azocarmine solution:
Grubler's azocarmine ..... 1 gram
Distilled water ..... 100 cc.
Heat, cool, and acidify with 1 cc. glacial acetic acid
(b) Aniline alcohol:
Aniline oil (C.P.) ..... 2 drops
95 per cent alcohol ..... 50 cc.
(c) Acid alcohol:
Glacial acetic acid ..... 2 drops
95 per cent alcohol ..... 100 cc.
(d) Toluidine blue:
0.5 per cent aqueous solution of toluidine blue, C.I. no. 925.
Filter just before use.

Note: Until a given new batch of stain has proven its strength it is well for the technician to cut a number of extra sections so as to avoid delay if the first stained preparations are not satisfactory in their minute detail. Material taken 12 hours postmortem will show some cellular degenerative changes but these should cause no significant difficulty in the classification of the cells.

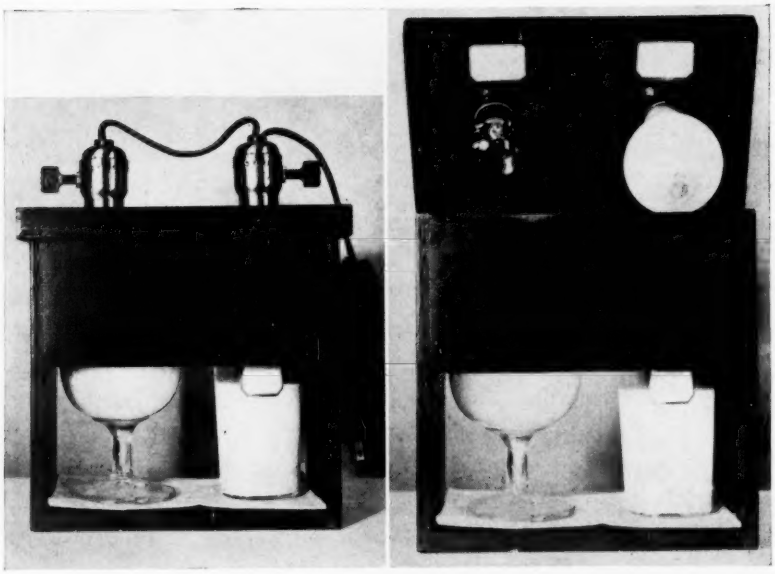
AN EFFICIENT PARAFFIN BATH*

C. W. MAYNARD
Pueblo, Colorado

A paraffin bath which is small, inexpensive, and which will not heat tissues above the melting point of the embedding material used, is of much advantage.

The idea of melting paraffin from above by the use of an electric light bulb, is, of course, old. The apparatus here described puts the idea into practical use. It consists of a box with a loosely-fitting cover in which are set two standard brass sockets far enough apart so that the bulbs in the sockets will

* Received for publication October 1, 1938.



A B

FIG. 1—A. PARAFFIN BATH

FIG. 1—B. PARAFFIN BATH

Cover tilted to show under surface

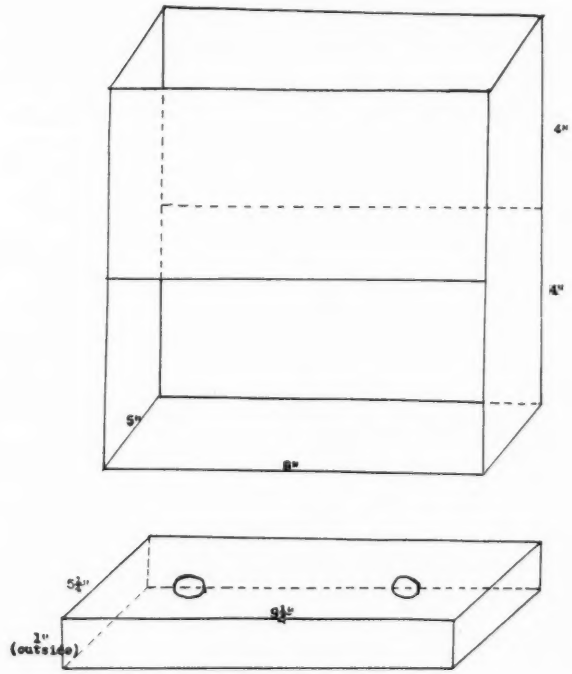


FIG. 2. PARAFFIN BATH

All measurements inside unless noted

fit into the tops of drinking glasses in the box. The sockets are wired together in parallel so that either may be turned on or off without disturbing the other. The lower half of each long side of the box is left open to permit inspection of the paraffin in the glasses.

One glass may contain the paraffin with a diluent (chloroform, toluol), the other the 56° paraffin.

With the bulbs lighted the paraffin melts down from the top, and the tissues, in porous or perforated containers, rest on top of the unmelted material. Here the temperature cannot be much above the melting point of the paraffin. The degree of heat is easily regulated by the use of bulbs of appropriate wattage.

The apparatus illustrated is made of $\frac{3}{8}$ inch plywood, and handles up to thirty tissue blocks at a time without crowding.

HEMATOLOGICAL STANDARDS*

FRED BOERNER

Considerable confusion has resulted from the multiplicity of hematological standards in the effort to determine the "100 per cent normal".

Numerous investigations have established the inherent variability of hematologic findings in varying ages, sexes and localities, though it is possible to establish an average normal *range*. A fairly satisfactory solution of the problem can be achieved by arbitrary normals and adapting them to the newer findings in the field of hematology.

For an arbitrary "100 per cent normal" hemoglobin 16.6 grams per cent is a satisfactory figure because it can be converted readily into percentage by multiplying by six.

The various means and indices can then be calculated as follows:

$$\textit{Color index.}\quad \frac{3 \times \text{gms. of Hb.}}{\text{1st 2 numbers of } RBC} = \text{C.I.}$$

* Received for publication November 7, 1938.

Abstract of a paper presented before The Technician's Institute, Temple University Medical College, Philadelphia, Penn.

Mean corpuscular hemoglobin. Color index × 33.3 = M.C.H. (micromicrograms).

$$\textit{Volume index.}\quad \frac{\text{cc. of packed cells per 100 cc.}}{\text{1st 2 numbers of } RBC} = \text{V.I.}$$

Mean corpuscular volume. Volume index × 100 = M.C.V. (cubic microns).

$$\textit{Saturation index.}\quad \frac{\text{C.I.}}{\text{V.I.}} = \text{S.I. or}$$

$$\frac{3 \times \text{gms. Hb.}}{\text{cc. of packed cells per 100 cc. blood}} = \text{S.I.}$$

Normal ranges. The normal range for the color and volume index when using the above methods is 0.8 to 1.00 and for the saturation index 0.9 to 1.1.

In the above, there is no reference to "100 per cent normal". When desired, findings in grams are readily converted into terms of per cent by multiplying the grams found by six.

Leukocytes. The leukocyte count should be reported in absolute numbers of each type of cell. This will show at a glance whether there is an increase or decrease of any type. This is very easily calculated by crossing off the last two figures of the total and multiplying by the percentage.

Normal ranges. The normal range for hemoglobin, cell counts, leukocytes are given in most of the more recent text books.

TOXICOLOGY IN A GENERAL HOSPITAL

A Note Concerning the Published Technique of Dale G. Friend

ALEXANDER O. GETTLER

Professor of Chemistry and Toxicology at Washington Square College, New York University

In the technical supplement of the July 1938 number of the American Journal of Clinical Pathology, there appeared a paper entitled "Toxicology in a General Hospital" by Dale G. Friend of the Department of Medicine of Harvard University Medical

School, and the Laboratories of Peter Bent Brigham Hospital, Boston, Mass. In this paper Friend outlines analytical procedures for ethyl alcohol, arsenic, barbital compounds, bromides, iodides, mercury, and morphine. The paper contains several misleading and fallacious statements. Since the methods in this publication will be read, accepted, and used by many internes and hospital technicians, attention ought to be called to the several misleading and erroneous statements made therein.

REGARDING ARSENIC

(a) When urine, or stomach contents containing arsenic, is concentrated by evaporation, as directed by Friend, most of the arsenic present, if not all, will be vaporized and lost as $AsCl_3$. This experimental fact is the basis of a quantitative method for the determination of arsenic. To separate the arsenic from interfering substances it is quantitatively distilled with a stream of HCl from an HCl solution. (Autenrieth and Warren, *Detection of Poisons*, 6th Ed., p. 509; also Scott, W. W., *Standard Methods of Chemical Analysis*, 4th Ed., vol. 1, p. 38.) The arsenic is then quantitatively determined in the distillate and not in the residue.

(b) Urine samples never contain nitric acid or free chlorine. If both nitrates and arsenic were taken by the subject, the nitrates excreted in the urine, will quickly, during the evaporation with the concentrated HCl, be reduced by the urinary components, and hence will not be capable of oxidizing the arsenic to the less volatile pentavalent form. If Friend had in mind the nitric acid and free chlorine, remaining from the oxidizing agents that he may have used for decomposing the organic components of the urine, he certainly does not give any indication of this in his paper. Furthermore the Reinsch test is always applied without any previous oxidation of the urine or stomach contents. (Webster, R. W., Legal Medicine and Toxicology, 1930, p. 507.)

(c) Friend claims that with the technique as described he can detect as little as 0.0065 mgm. of arsenic in urine or stomach contents. The highest sensitivity obtainable with the Reinsch-Volatilization test, in pure aqueous solution is 1 part in 400,000 (Rosenthaler, L., Toxikologische Mikroanalyse, 1935, p. 52).

This means 0.025 mgm. in a 10 cc. volume. The statement in Webster's *Toxicology* 1930, p. 509, that Wormley was able to detect 0.0013 mgm. of arsenic by this method is misleading. Wormley (*Micro-chemistry of Poisons*, 2nd Ed. 1885) tried out the sensitivity of the Reinsch-Volatilization Method, by using a very small volume (*few drops*) of a *pure aqueous solution* of arsenic. In this work Wormley did not use large volumes of urine or stomach contents, nor did he attempt any evaporation of the solution, such as described by Friend. In the case of urine or stomach contents the Reinsch test is about ten times less sensitive, and if one attempts to concentrate the urine by evaporation, as Friend advises, without previously making it alkaline, an arsenic containing urine may give an entirely negative Reinsch test.

METHOD FOR ARSENIC

The following technique should be followed when applying the Reinsch test: 200 cc. or more of urine, are made alkaline with NaOH, and evaporated to about 40 cc. The concentrated urine is then acidified with HCl and the Reinsch test applied as directed in Friend's paper. Evaporation of the urine as such, or evaporating the urine after acidifying causes a loss of any arsenic present due to the volatility of the $AsCl_3$. When doing the Reinsch test, decomposition of the organic material present in the urine or stomach contents by oxidizing agent is unnecessary and never resorted to. (Webster, R. W., *Legal Medicine and Toxicology*, 1930, p. 507.)

REGARDING MORPHINE

The technique given by Friend for the detection of morphine follows: Evaporate the urine or stomach contents (he does not say what volume of either should be used) to dryness in a white porcelain evaporating dish. With a glass rod transfer a drop of the sulfuric acid-formalin reagent to the dried residue. If morphine is present an intense purple-red color changing first to violet and then to a pure blue develops. A marked reaction is given by as small an amount as 0.02 mgm. morphine.

The Marquis reagent (sulphuric acid-formalin reagent) is a very sensitive test for morphine, when the latter is dry and relatively pure. Before the test can be applied it is essential that the morphine be first extracted from the urine or the stomach contents, and then put through a series of purifying procedures by means of immiscible solvents. This technique of purification can be found in any elementary textbook on toxicology. Friend entirely ignores the extraction procedure. He recommends evaporation of the urine (containing as little as 0.02 mgm. of morphine) to dryness, and to the conglomerate of urine residue, which may weigh several grams, and has a brown to dark brown color, he adds one drop of the Marquis reagent and claims that he can see the color changes attributed to morphine. It is well-known that components of tissues, urines and stomach contents interfere or may entirely mask this reaction, that the dried urine residue has a dark brown color, and that the concentrated sulphuric acid of the Marquis reagent chars and blackens the urine residue. It is unbelievable therefore that anyone could see the play of colors, purple violet to pure blue produced by 0.02 mgm. of morphine mixed with 3 grams of urine residue (from 50 cc. of urine). Even if one had 10 mgm. of morphine in 50 cc. of urine (equivalent to 300 mgm. or 5. grains of morphine in the 24 hour sample), the play of color produced by morphine would be inhibited and could not be seen if one followed Friend's technique.

METHOD FOR MORPHINE

The following technique should be followed when testing for morphine. The urine, or stomach contents (if not acid) are acidified with hydrochloric or tartaric acid, and then evaporated down to a syrup in a porcelain evaporating dish on the steam bath. To this residue, ten times its volume of 95 per cent alcohol is added gradually and with constant stirring. After allowing to stand for about one hour, the material is filtered through a dry filter paper. The filtrate is evaporated to a thick syrup, and again treated with ten times its volume of alcohol, allowed to stand and then again filtered through a dry filter paper. The filtrate is evaporated to a thick syrup, to free it of all alcohol. The residue is then treated with about 50 cc. of hot water, thoroughly stirred, and then filtered. The filtrate, when cool, is now extracted in a separatory funnel with two successive 50 cc. portions of ether. The two ether extracts are discarded.

The aqueous layer is now made alkaline with NaOH and again extracted in a separatory funnel with two successive 50 cc. portions of ether. In the case of morphine analysis these two ether extracts are also discarded, because morphine is not soluble in ether from alkaline (NaOH) solution. The aqueous layer is now acidified with sulphuric acid, warmed to about 60°C. in order to expel any residual ether, and then made slightly alkaline with ammonium hydroxide, and twice extracted with 50 cc. portions of hot chloroform. The two chloroform layers are combined and washed with a little water by shaking in a separatory funnel. The chloroform layer is now evaporated to dryness on water bath. To further purify the extracted morphine, the residue is dissolved in about 20 cc. of water slightly acidified with sulphuric acid, and filtered if necessary. The aqueous solution is made slightly alkaline with ammonium hydroxide and twice extracted with 20 cc. portions of hot chloroform. The two chloroform layers are combined and washed by shaking in a separatory funnel with a little water. The chloroform layer is next filtered through a dry filter paper and evaporated to dryness on water bath. The residue will contain the morphine in a fairly pure state. The test described by Friend is applied to this purified residue.

COMPARATIVE STUDY OF THE MAZZINI FLOCCULATION AND THE KLINE EXCLUSION TEST*

ALFRED S. GIORDANO, AGATHA M. WILHELM AND BONITA G. CARLSON

From the South Bend Medical Laboratory, South Bend, Indiana

In the past few years a number of flocculation tests for the diagnosis of syphilis have appeared in the medical literature, many of which, after a brief trial, have been discarded because they offered no advantage over those already in use and of proved value.

One of the more recent contributions in this field is by L. Y. Mazzini[1]. Because of the simplicity of the procedure and the easily read and clear cut results, the test was given a trial and applied to a series of six thousand (6,000) blood sera in our laboratory.†

The Kline Exclusion test was run simultaneously on all sera included in this series. The Kline test, as performed in our laboratory, was rated in the second study conducted by the Committee on Evaluation of Serodiagnostic Tests for Syphilis

at top of page 81.

* Received for publication January 13, 1939.

† The antigen and directions for the performance of the test have been furnished through the courtesy of Mr. Mazzini.

Sensitivity, 92.7 per cent, as compared with the control, 88.8 per cent.

Specificity, 98.06 per cent, as compared with the control, 99.03 per cent.

The blood sera studied in this series were taken from the following sources: 1. Four regional hospitals. 2. The private practice of local physicians. 3. Local U.S.P.H. Clinic. 4. Routine compulsory examinations of two local industrial corporations. The last group comprised 2,155 or 35.91 per cent of the entire series.

Analysis of 6,000 sera by Mazzini and Kline tests

	TOTAL NUMBER	PERCENTAGE
Complete agreement	5,707	95.12
Mazzini and Kline, negative	4,917	81.95
Mazzini and Kline, strongly positive (3+, 4+)	346	5.77
Mazzini and Kline, weakly positive (1+, 2+)	444	7.40
Partial agreement	78	1.30
Kline, strongly positive (3+, 4+), Mazzini, weakly positive (1+, 2+)	14	0.23
Mazzini, strongly positive (3+, 4+), Kline, weakly positive (1+, 2+)	64	1.07
Complete disagreement	215	3.58
Mazzini, weakly positive (1+, 2+), Kline, negative	78	1.30
Mazzini, negative, Kline weakly positive (1+, 2+)	132	2.20
Mazzini, strongly positive (3+, 4+), Kline, negative	2	0.03
Mazzini, negative, Kline, strongly positive (3+, 4+)	3	0.05

The most interesting group is that of complete disagreement. Each case in the group was investigated and the clinical history evaluated in regard to syphilis. This group includes two hundred fifteen (215) cases (3.58 per cent).

Analysis of complete disagreement series

	NUMBER OF CASES	PERCENTAGE
Mazzini, falsely positive	37	0.62
Kline, falsely positive	100	1.66
Mazzini, truly positive	44	0.73
Kline, truly positive	21	0.35
Mazzini, doubtful	5	0.08
Kline, doubtful	8	0.13

DISCUSSION

From the above figures, it is obvious that the Mazzini Flocculation test compares favorably with the Kline Exclusion test in specificity and sensitivity. Technically, it has decided advantages. The negative tests are clear cut and more easily read than the Kline. The preparation of the antigen emulsion is simplified by the use of buffered saline solution, thus eliminating the frequent checking of the pH, which is necessary in the Kline test. The antigen emulsion is ready for use after fifteen (15) minutes of ripening at ice box temperature, a factor of importance when testing blood for emergency transfusions. Because of the similarity of procedure, the Mazzini test adapts itself as a parallel test to the Kline, involving a minimum expenditure of time, equipment and material.

SUMMARY AND CONCLUSIONS

1. A comparative study of the Mazzini Flocculation and the Kline Exclusion tests on six thousand (6,000) blood sera is reported with the following results:

(a) Complete agreement of both tests occurred in 5,707 or 95.12 per cent of the cases.

(b) Partial agreement occurred in 78 or 1.30 per cent of the cases.

(c) Complete disagreement occurred in 215 or 3.58 per cent of the cases.

2. Analysis of the complete disagreement series, after a clinical history was taken, proved 100 or 1.66 per cent of the Kline tests to be falsely positive and 21, or 0.35 per cent, to be truly positive. Similarly, 37, or 0.62 per cent of the Mazzini tests proved to be falsely positive and 44, or 0.73 per cent, to be truly positive.

3. The Mazzini Flocculation test manifests slightly greater specificity and sensitivity than the Kline Exclusion test in the laboratory diagnosis of syphilis.

4. The Mazzini Flocculation test offers a rapid and simple test for the diagnosis of syphilis without sacrificing sensitivity or specificity.

5. In a series of 6,000 cases, 1.08 per cent of the true positives would have been missed had not at least two tests been run on the same blood.

REFERENCE

(1) MAZZINI, L. Y.: Slide Flocculation Test for Syphilis. In press. The American Journal of Clinical Pathology.

PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

A METHOD FOR THE DARK FIELD EXAMINATION OF PUS FOR SPIROCHAETA PALLIDA

LEON FRIEDMAN, Jour. A. M. A., **112:** 134, January 14, 1939

Pus or bloody serum from urethral discharge or from the suspected lesion is collected in fine straight capillary tubes 12 cm. in length which are sealed at one end by a small flame. The tubes are placed in receptacles made of glass tubing 6 mm. in outside diameter, 13 cm. in length and sealed at one end, and centrifuged at 1,000 revolutions per minute for 10 minutes. The tubes are filed and broken just above the line of separation of the sediment from the clear supernatant serum which is expelled onto a slide by pressure from a small rubber bulb. Examination is then made with the dark field microscope. The organisms have been found in considerable numbers by using this method when the examination of the material without centrifuging was negative.

ARTIFICIAL CONCENTRATION OF TEST SERUMS IN BLOOD GROUPING

M. C. TERRY, Jour. A. M. A., **112:** 135, 1939

Typing serum which falls below the desired titer can be concentrated by the simple and inexpensive expedient of putting it in a cellophane dialysis tube ¾ inch in diameter and placed in front of an electric fan. In one example a 16 cm. column exposed to the current of a 16-inch fan for 2½ hours went down to 4 cm. The agglutination was concentrated proportionately.

The author comments on the advantages of this method over the lyophile methods.

A METHYLENE BLUE STAIN FOR SECTIONS OF FORMALIN-FIXED MARROW

HAROLD GORDON, J. Lab. & Clin. Med., **24:** 405, 1939

The method depends on the simple principles of deformalization, sensitization, overstaining and selective differentiation.

Method: Fix in 10 per cent formalin, decalcify in a dilute mineral acid, wash, dehydrate in alcohol, clear in xylol and embed in paraffin. After sectioning (4–6 microns) sections are affixed to slides and dried in the oven, decerated and brought into water in the usual manner.

Deformalization: The formalin is removed by immersing the sections in 10 per cent aqua ammonia (one part 28 per cent ammonia and 9 parts of water) one minute.

Sensitization: Wash sections in water to remove ammonia and mordant for three minutes in a mixture consisting of equal parts of Zenker's fluid (without acetic acid) and saturated aqueous solution of copper sulfate.

Staining: After thorough washing in water, stain sections one and a half to three minutes in the following mixture; 1 per cent aqueous solution of Eosin Y—one part; 1 per cent aqueous solution of phloxin—three parts. Wash in water and stain four minutes in Loeffler's alkaline methylene blue heated to 37°C.

Differentiation: Wash in water and differentiate in absolute alcohol. Control by microscopic examination. Clear in xylol and mount in balsam.

THE PREPARATION OF PLASTER OF PARIS EMBEDDING BOXES

A. N. Solberg, Stain. Tech., **14:** 27, 1939.

Cut pieces of paraffin of the size you desire for blocks. Bevel the block about 15° so that the bottom will be smaller than the top. Seal the block, wide side down, to a flat surface (copper plate or cardboard) and place around it two embedding angles about one eighth inch from the widest part of the block. Coat the paraffin block with vaseline. Fill the mold thus formed with plaster of Paris mixed with water. Allow the plaster to dry for an hour and remove the embedding angles. When completely dried, lift the plaster box off the paraffin block and trim with a knife and sandpaper.

To use the box, soak in water, then fill with melted paraffin, drop in the tissue and cool as with paper or cellophane boxes. The paraffin block thus formed shrinks and floats free.

THE DETECTION OF ACETONE AND ACETOACETIC ACID IN URINE

Jonas Kamlet, J. Lab. & Clin. Med., **24:** 206, 1938

The author points out the undesirable features of strong ammonia as a reagent in the usual tests for acetone and acetoacetic acid and suggests the substitution of a strongly alkaline organic amine, monoethanolamine.

The Procedure: 1. Place 10 cc. of urine in a test tube. 2. Add 1 drop (0.05 cc.) of a saturated aqueous solution of sodium nitroprusside and 1 cc. of a 15 per cent aqueous solution of monoethanolamine and mix.

In the presence of acetone or acetoacetic acid the entire contents of the tube will turn to a deep permanganate-violet within sixty seconds. The depth

of the color is in proportion to the concentration of the substances in question. The procedure will detect 0.054 per cent of acetone in the urine. The normal range of creatinine concentration gives no color while a saturated solution of creatinine gives a brownish-orange color which can hardly be mistaken for the deep permanganate-violet given by acetone and acetoacetic acid.

TRICHINOSIS IN CLEVELAND

C. H. Evans, Jr., Jr. Inf. Dis., **63:** 337, 1938.

The author cites figures from various workers showing autopsy incidence of trichinosis of 13.6 to 27.6 per cent, all of which are much lower than his own figure of 36 per cent obtained in an examination of the muscle from 100 consecutive autopsies. The discrepancy is explained by the fact that not only was the diaphragm (the usual muscle used in these studies) but the intercostal muscles and sterno mastoid as well were examined; moreover, he used two methods of examining the tissues, (1) that of compressing a gram of muscle between two glass plates and examining microscopically, and (2) the digestion-Baermann method. The organisms were found twice as often by means of the digestion method as with the microscopic.

In his series the diaphragm alone showed the worms in 26 cases whereas the intercostal alone showed them in 21 cases and the sterno mastoid alone in 1 case.

From this study, it seems clear that in future studies of this condition, examination of the intercostal as well as the diaphragm must be done and the two methods of examination, microscopic and digestion, must be carried out in order to obtain anything like a correct figure.

ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

METHOD FOR AMYLASE DETERMINATION

To the Editor: Please advise where I can find a reliable method for amylase determination. The viscometer method is unsuitable in my present location because of expense. P. J.

Answer: The method of Somogyi is recommended. (Somogyi, M., Micromethods for the Estimation of Diastase, J. Biol. Chem., **125:** 399, 1938.) The procedure consists in incubating a starch solution with 1 cc. of plasma, serum or whole blood. After thirty minutes, the reaction is stopped and the protein precipitated by adding definite amounts of copper solvent and sodium tungstate. The supernatant fluid after centrifuging is analyzed for reducing sugar by the Shaffer-Hartmann technique.

Correction is made for the glucose content of the plasma. The results are expressed in units. Thus a diastase value of 120 signifies that under the standardized conditions described by Somogyi, 100 cc. of plasma would produce from starch, cleavage products having the same copper-reducing power as 120 mg. of glucose. The normal diastatic activity ranges from 80 to 150 units.

DEMONSTRATION OF TRICHINAE IN MUSCLE

To the Editor: 1. Will you give me a method whereby trichina larvae after gastric digestion can be mounted on a slide so as to keep for a year or so and be of no danger to handlers of the slide?

2. Is there any simple method whereby the bits of muscle and diaphragm of infected rats pressed under a cover-slip can be preserved for the same length of time? A. E. T.

Answer: For the preservation of larvae after digestion of the muscle in artificial gastric juice, Bogg's method may be used. Place the larvae in a mixture of glycerin (20 per cent) and alcohol (80 per cent) which both fixes and clears the organisms. Allow them to remain in this fluid in a partially covered jar until the alcohol has completely evaporated. Transfer to a glass slide, remove the excess glycerin with blotting paper, mount in glycerin jelly, and after this has hardened, seal the cover with microscopic cement.

2. Place bits of muscle in some glycerin preservative, such as 50 per cent glycerin or, as Simon recommends, glycerin containing a concentration of 5 per cent acetic acid, or perhaps better still in Lacto-phenol prepared as follows: Glycerol, 2 parts; Phenol, 1 part; Lactic acid, 1 part; Distilled water, 1 part. The tissue may be left in any of these indefinitely. When pressed between glass slides or a muscle compressor, the demonstration of the worms is beautiful. For permanent preparations, mount as in Bogg's method.

For general demonstration purposes, fixation in formaldehyde and embedding in paraffin is satisfactory, but for fine histological details it is recommended that the muscle be fixed in acetic-corrosive sublimate solution; saturated aqueous solution of corrosive sublimate to which is added immediately before using sufficient glacial acetic acid to make a concentration of 5 per cent. Small bits are placed in this for 24 hours and washed in running water for 12 hours. Iodine treatment for the removal of the sublimate may be carried out either before embedding and sectioning or afterward. Stain with hematoxylin and eosin or other general stain.

SIMPLE METHOD FOR HOLDING SEDIMENTATION TUBES

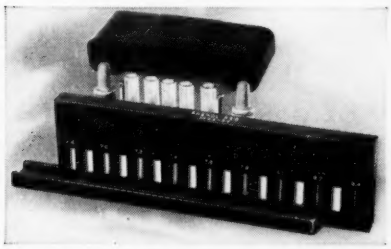
E. E. Myers, M.D., Myers Clinic Hospital, Philippi, W. Va.

Plasticine has been recommended[1] for holding sedimentation tubes in the upright position during reading of sedimentation tests. A very satisfactory base for the plasticine is an ordinary porcelain plate with concavities as used for color reactions. The type with deep concavities works better. The depressions are filled with the plasticine either level or slightly rounded and the tubes pushed down in these. The tubes should be vertical and this position is probably closely enough approximated by lining them up with parts of the building or furniture as, say, a wall and window casing.

REFERENCE

(1) Nicholson: Laboratory Medicine. Lea & Febiger, 1934.

NEW pH SLIDE COMPARATOR



A new Slide Comparator for the colorimetric determination of pH, chlorine and phosphates has been developed by W. A. Taylor & Co., 872 Linden Avenue, Baltimore, Md.

The new outfit is molded entirely from plastic. Radical changes in design have resulted in marked improvement in appearance, durability and ease of handling. The weight has also been considerably reduced. All pH, chlorine and phosphate values, as well as the indicator names, are engraved in white directly on the plastic slides. Improved catches are used to hold the top on the base and all metal parts are rustproof. The whole outfit, including the slide is 10 in. long, $2\frac{1}{8}$ in. wide and 4 in. high and weighs only $1\frac{1}{2}$ lbs.

The new Comparator consists of a slide and a base. Each slide contains 9 color standards alternating with ampoules of distilled water. All color standards are guaranteed by the manufacturer to maintain their accuracy for a period of 5 years.

The base contains 2 vials of indicator solution, with 0.5 cc. pipettes, 5-5 cc. test tubes, and a piece of etched glass in a special compartment. Determinations are made by filling three of the test tubes with the test sample, adding 0.5 cc. of indicator solution to the middle one, placing the slide on the base and moving it back and forth until the test sample matches one of the color standards. The pH, chlorine or phosphate value is then read off directly from the values on the slide. One base can be used with any number of color standard slides. Full information on these outfits can be obtained from the manufacturer.

CORRECTION

Dr. E. E. Myers, whose note upon "A Simple And Efficient Method for Closing Blood Pipettes" appeared in the January, 1939 issue (page 42) writes as follows:

I would like to make a correction in my article.

The last paragraph is a caution regarding losing of fluid from the pipette. This, to my chagrin, I have only discovered today is due to a fault in my technique in closing the pipette. If the tube is stretched, slightly, perpendicularly to the pipette as it is brought around, no movement in the pipette fluid will

occur; whereas, if it is brought around loosely and then stretched parallel to the pipette (as I have been doing), it kinks, air is compressed and a small amount of fluid is forced from the pipette.

This paragraph should read:

As in any similar method of closure, the tubing should be stretched perpendicularly to the pipette as it is brought around to seal the large end, otherwise a small amount of fluid may be lost.